CHAPTER IV

RESULTS

4.1. Microcephaly

4.1.1. General description

This study found that from a previous study of 527 individuals with MR, 48 (23 female and 25 male) unrelated individuals were identified as being microcephalic with a head circumference ranging from -2 to -4 SD, which counted for 9.1% of all the collected MR individuals. The ages of all the individuals were between 7 and 32 years**.**

Below are the demographic data of the mentally retarded individuals with microcephaly (Table 10).

Table 10. Demographic data of mentally retarded individuals with microcephaly

Characteristics	N	%
Age (years)		
<14	24	50
>14	24	50
Gender		
Female	23	47.9
Male	25	52.1
Epilepsy	7	14.6
OFC		
Between -2SD and -3SD	6	12.5
Between -3SD and -4SD	22	45.8
<-4SD	20	41.7

DNA sequencing was performed on all 48 samples of the MR individuals with microcephaly. Analysis for *ASPM* gene mutations was performed, followed by analysis of *WDR62* gene. Only those patients with head circumference less than -4SD underwent sequencing of *MCPH1, CDK5RAP2, CENPJ* and *STIL* gene.

4.1.2. *ASPM*

Sequencing was performed on 48 samples for *ASPM* gene. After PCR amplification and DNA purification, the amplicons were sequenced using 42 sets of PCR primers. Sequences were compared with the reference genomic and cDNA sequences (NM_018136.4).

Analysis of the gene showed the various variants found on the individuals, and the variants that were not listed as common polymorphism were further examined using protein prediction program to determine the likelihood of pathogenicity (Table 11).

Table 11. Study subjects with genetic variants of *ASPM* gene

No	Patient *ASPM*	Variants	Homo-zygosity	Protein	Class	Polyphen prediction	Gran tham score (0-215)	Align-GVGD predict ion	SIFT prediction
1	DNA10-18704	c.2026+15_2026+20del		p.?	UV2	NA	NA	NA	NA
2	DNA10-19851	c.249G>T	Homo	p.=	UV2	NA	NA	NA	NA
3	DNA11-00132	c.489A>G	Het	p.=	UV2	NA	NA	NA	NA
		c.937A>G	Het	p.Ile313Val	UV3	NA	29	C0	NA
4	DNA11-00126	c.73C>G	Het	p.Arg25Gly	UV3	0,827 (possibly damaging)	125	C0	NA
		c.1617A>G	Het	p.=	UV2	NA	NA	NA	NA
5	DNA11-01705	c.582T>C	Het	p.=	UV2	NA	NA	NA	NA
		c.4123A>G	Het	p.Ile1375Val	UV2	NA	29	C0	0,26 (tolerated)
6	DNA11-01706	c.9539A>C	Het	p.Gln3180Pro	UV3	0,969 (probably damaging)	76	C0	0,15 (tolerated)
7	DNA11-01707	c.582T>C	Het	p.=	UV2	NA	NA	NA	NA
		c.4123A>G	Het	p.Ile1375Val	UV2	NA	29	C0	0,26 (tolerated)
8	DNA11-01710	c.1583A>G	Het	p.His528Arg	UV2	0,004 (benign)	29	C0	0,40 (tolerated)
9	DNA11-01712	c.2378G>A	Het	p.Arg793Gln	UV2	0.211 (benign)	125	C0	0,10 (tolerated)
10	DNA11-02676	c.7114A>G	Het	p.Arg2372Gly	UV2	0,992 (probably damaging)	125	C0	0,18 (tolerated)
		c.8452G>T	Het	p.Ala2818Ser	UV3	0.987 probably damaging	99	C0	0.09 (tolerated)
11	DNA10-19850	c.4029G>A	Het	p.=	UV2	NA	NA	NA	NA
12	DNA11-01709	C.844A>C	Het	p.Asn282His	UV3	0.927 Probably damaging	68	C0	0.06 (tolerated)
		c.905G>A	Het	p.Cys302Tyr	UV3	0,003 benign	194	C0	0,10 (tolerated)
		c.1007C>A	Het	p.Thr336Lys	UV3	0.889 probably damaging	78	C0	0.35 (tolerated)
		c.5579C>T	Het	p.Ala1860Val	UV3	NA	64	C0	0,08 (tolerated)
		c.7787T>C	Het	p.Val2596A	UV3		64	C0	0.79 (tolerated)
		c.8166T>C	Het	p.=	UV3	NA	NA	NA	NA
13	DNA11-01713	c.9492T>C	Het	p.=	NA	NA	NA	NA	NA

NA: Not available; C0: Class 0 on Align-GVGD prediction programme; Homo: homozygous; Het: heterozygous; p.= : synonymous amino acid change; UV2 (Unclassified variant 2): “unlikely to be pathogenic”; UV3 (Unclassified variant 3): “likely to be pathogenic”.

One subject, sample code DNA11-02676, was further examined due to the concurrent presence of 2 unknown heterozygous variants in *ASPM* gene, c.7114A>G and c.8452G>T, each with high Grantham scores of 125 and 99 respectively (Figure 10). Based on this finding, follow up was done and samples were requested from the parents and at least available two siblings. The samples from the family (father, mother, brother, twin sister) were sequenced and analysed for the presence of the unknown variants (Figure 10).

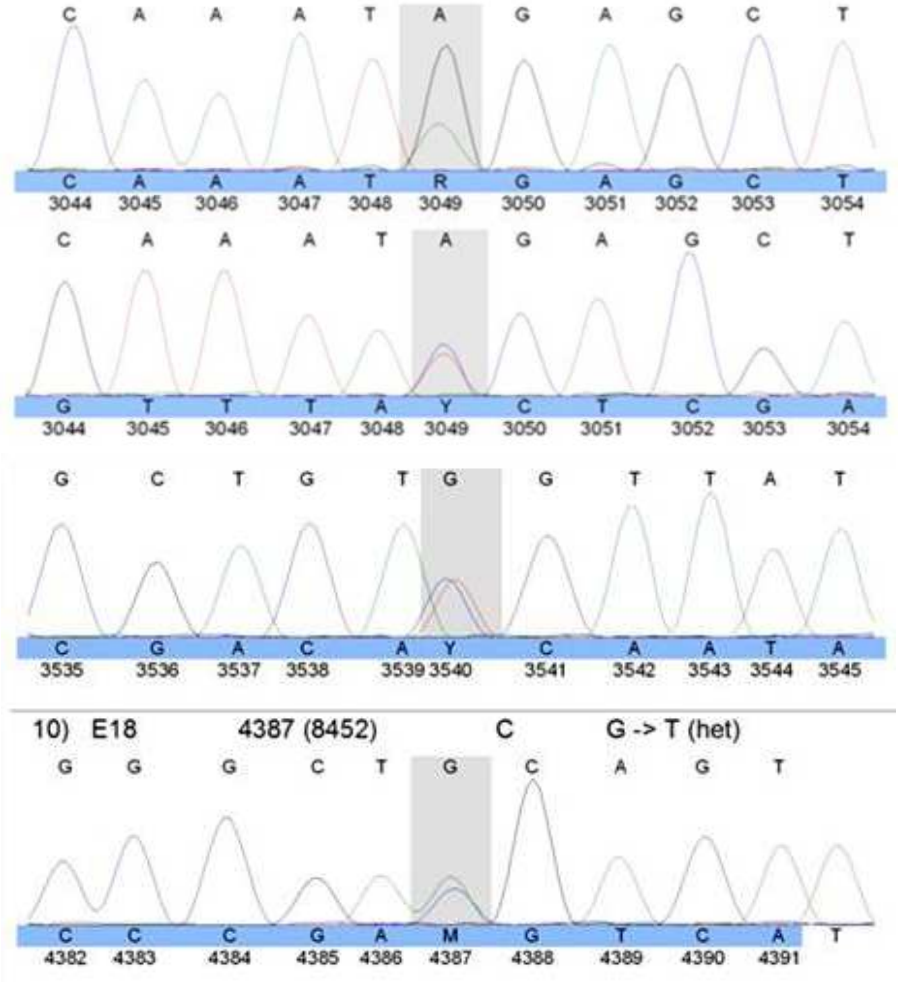

Figure 10. Sequencing result of subject DNA 11-02676. Variant c.7114A>G and c.8452G>T are shown in the forward and reverse sequence.

Polyphen-2 protein prediction for the 2 variants of subject DNA11-02676 with high scores and are shown (Figure 11, Figure 12).

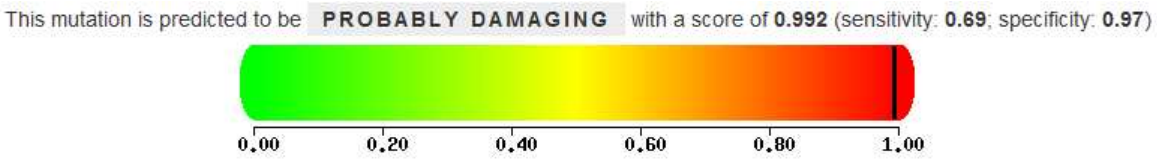

Figure 11. Polyphen-2 result for variant c.7114A>G of subject DNA11-02676. Polyphen-2 scores at 0.992, predicted as probably damaging.

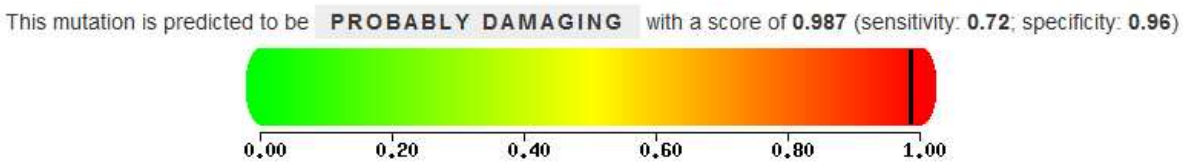

Figure 12. Polyphen-2 result for variant c.8452G>T of subject DNA11-02676. Polyphen-2 scores at 0.987, predicted as probably damaging.

Figure 13 shows the pedigree of of subject DNA11-02676 after follow up DNA collection of 2 parents and 2 siblings.

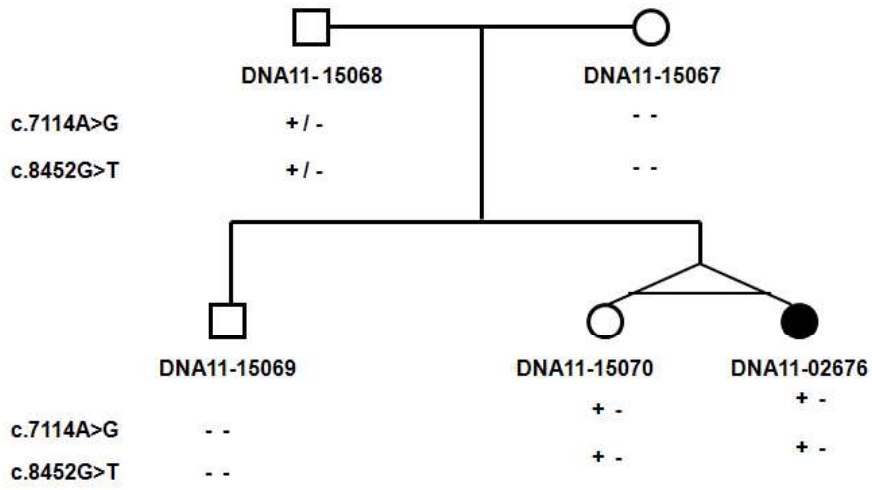

Figure 13. Pedigree of subject DNA 11-02676 follow up. (+): Variant is present, (-): Variant is not present.

The results from the family (Figure 13) showed that both variants were identified in the father and none in the mother, indicating that the 2 variants originated from the same allele in the father. As MCPH is an autosomal recessive disease, it is not likely that these variants were the pathogenic cause since the father was unaffected.

As the twin sister also had the exact 2 variants, there was still a possibility that there was a second hidden mutation from the maternal allele which caused the pathology in subject DNA11-02676 but not the twin sister. Identifiler test was subsequently performed to determine the zygosity status of the twins with the result that the twins were monozygotic (identical common variants were found in 15 marked locations indicating identical DNA sequence). The twin sister did not have any dysmorphic feature or mental retardation. This monozygosity status further strengthen the argument that autosomal recessive inheritance for the mental retardation and microcephaly in this family was unlikely.

4.1.3. *WDR62*

WDR62 gene sequencing and analysis was performed on 48 samples, using a set of 27 PCR primer pairs. Sequences were then compared with the genomic reference (NM_001083961.1). As with *ASPM,* only UV2 (unlikely to be pathogenic) or UV3 (likely to be pathogenic) results were further analyzed using protein prediction programs while UV1 (certainly non-pathogenic) variants were not included in this list (Table 12).

Table 12. Study subjects with genetic variants of *WDR62* gene

No	Patient WDR62	Variants	Homo-zygosity	Protein	Class	Polyphen prediction	Grantham score (0-215)	Align-GVGD prediction	SIFT
1	DNA11-01705	c.2418T>G	Het	p.Cys806Trp	UV2	0.974 (probably damaging)	215	C0	0.08 (tolerated)
2	DNA11-00116	c.4187G>A	Het	p.Arg1396His	UV2	NA	29	C0	0,68 (tolerated)

4.1.4. *MCPH1, CDK5RAP2, STIL* and *CENPJ*

MCPH1, CDK5RAP2, STIL and *CENPJ* gene analysis were performed. There were no UV2, UV3, UV4 variants found on these 4 genes and only UV1 single nucleotide polymorphisms (SNP) were found on these genes (Table 13). A list of all SNP found on the MCPH genes are available (Appendix IV), together with frequency comparisons with the European population. As SNP frequency data from the Indonesian population is still rare, a list of SNP in the Indonesian population may assist in future genome-wide association studies.

Table 13. List of *MCPH1, CDK5RAP2, CENPJ* and *STIL* variants found

Gene	Reference sequence	Findings
MCPH1	NM_024596.3	15 UV1 SNP found
CDK5RAP2	NM_018249.4	14 UV1 SNP found
CENPJ	NM_018451	6 UV1 SNP found
STIL	NM_001048166	3 UV1 SNP found

4.2. Macrocephaly

4.2.1. General finding

Below are the overview of characteristics (Table 14) of the mentally retarded individuals with macrocephaly. There were no unknown variants or SNP found in DNA sequencing and analysis of the PTEN gene in the 10 individuals of 527 mentally retarded individuals (1.9 %).

Table 14. Demographic data of mentally retarded individuals with macrocephaly

Characteristics	N	%
Age (years)		
<14	2	20
>14	8	80
Gender		
Female	2	20
Male	8	80
Epilepsy	0	0
OFC		
Between -2SD and -3SD	5	50
Between -3SD and -4SD	0	0
<-4SD	5	50